PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1017PC1	**FOR FURTHER ACTION** See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/DK 03/00266	International filing date *(day/month/year)* 22.04.2003	Priority date *(day/month/year)* 19.04.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/00		
Applicant BIOIMAGE A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 13.10.2003	Date of completion of this report 24.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Pellegrini, P Telephone No. +49 89 2399-3929

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00266

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-87 as originally filed

Claims, Numbers

1-75 as originally filed

Drawings, Sheets

1/23-23/23 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 73,75

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 73 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 75

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-72,74
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-72,74
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-72,74
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claim 73 is technically not supported and does not contain an essential technical feature of the invention, i.e. the fact that the two conjugates are present in different cell locations. For this reason, an assessment of novelty and inventive step will not be carried out.

2. Claim 75 does not contain technical features at all, therefore a search has not been carried out for such claim and no examination will be performed.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents are referred to in this communication:

   D1: HU CHANG-DENG ET AL: 'Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation.' MOLECULAR CELL, vol. 9, no. 4, April 2002, pages 789-798

   D2: WO 00 73437 A (MERCK FROSST CANADA INC ;XANTHOUDAKIS STEVEN (CA); CASSADY ROBIN () 7 December 2000

2. The subject-matter of claims 1-72 and 74 is novel (Article 33(2) PCT).

   The cited prior art does not disclose:

   a) a cell comprising two conjugates, each comprising an interacting protein and a terminal fragment of a complementation protein, wherein the two conjugates have different cellular locations (claims 1-60);

   b) methods for detecting protein interactions or protein translocation employing the cell system of a) (claims 61-72);

   c) a method for determining caspase activity employing a cell system as in a)

(claim 74).

D1 discloses an in-vivo complementation assay based on YFP reassembly to detect protein-protein interactions, the intracellular localization where such interaction takes place, and the translocation of the reconstituted complex. YFP was split into the two non-fluorescent terminal fragments YN and YC; the fragment YC was fused to the transcription activator ATF2, to give ATF2YC, and the fragment YN was fused to the protein Jun, to give JunYN. The intracellular localization of the interacting proteins (i.e. of the heterodimers ATF2YC-JunYN) in the perinuclear region of the cell was detected as a fluorescence emission localized in that region. Overexpression of the protein p38 determined translocation of the heterodimers to the nuclear region (page 792, left column, paragraph 3).

D2 (abstract; claim 4) discloses a method for determining caspase activity, comprising detecting caspase-induced decrease in free resonance energy transfer between two green fluorescent proteins (GFPs) linked with a peptide comprising a caspase cleavage site.

3. The subject-matter of independent claim 1 is inventive (Art.33(3) PCT).

a. D1, representing the closest prior art, discloses a cell comprising:
   (i) a first conjugate, comprising a first protein and the N-terminal fragment of a complementation protein, and
   (ii) a second conjugate, comprising a second protein and the C-terminal fragment of a complementation protein.

b. The difference between the cell of claim 1 and that of D1 is that, in the cell of claim 1, the first conjugate has a predominant location in a different cellular compartment from the second conjugate. The technical effect of this difference is that the cell of claim 1 is suitable for detecting protein translocation and for identifying modulators of protein-protein interactions. In the latter case, it is necessary that the two conjugates be present in different cellular compartments before the test compound is added, as otherwise the two fragments of the complementation protein would irreversibly bind to each other in absence of the modulator, and the successive addition of the modulator would not be effective in separating the complex. The objective technical problem of the present application in view of the closest prior art is therefore to adapt the system of D1 to the

detection of protein translocation and the identification of modulators of protein-protein interactions (with potential applications in drug screening). The solution proposed is a cell comprising two protein conjugates, wherein the predominant cellular locations of the conjugates are different from each other. This solution is not obvious to the skilled person, as there is no suggestion or indication in the prior art that would prompt the skilled person to modify the cell system disclosed by D1 in order to render it suitable for drug screening.

3.1. Being inventive claim 1, claims 2-60 dependent thereon are also inventive.

3.2 Claims 60-72 and 74, related to methods making use of the cell system of claims 1-60, are also inventive.

4. It si further noted that the function of the "first protein" of claim 5 is unclear (Art.6 PCT).